secretory mechanism requires the presence of CFTR protein which, in turn, is activated by the interaction with SLC26A3. The main defect in CF has long been thought to be the reduced epithelial chloride transport. However, recent works turned this theory on its head, showing that the primary defect in cystic fibrosis is the inability to activate bicarbonate-driven fluid secretion caused by chloride-bicarbonate exchange. This mechanism requires the involvement of both SLC26 and CFTR intact proteins: they form a unique complex by a direct interaction between SLC26A3 "STAS-like" and CFTR "R" domains and by an indirect interaction between PDZ domains of both proteins connected by the "PDZ-Binding Protein".
Aim: The aims of the study were to evaluate if mutations in genes encoding some SLC26 proteins (all characterized by the STAS-like and PDZ domains) may reduce the the interactions between SLC26 genes (A3, A6, A7, A8 and A9) and CFTR proteins, thus reducing the activation of CFTR. In other words mutations of SLC26 genes might be causative of CF.
Patients and methods: We analyzed, by direct sequencing, 25 patients bearing CF and 25 patients bearing CFTR related disease, with one or both undetected CFTR mutations. Furthermore, 100 non CF alleles were analyzed as controls.
Results and conclusions: For SLC26A3 we identified two mutations and two intronic variants in CF patients. Similarly, for the other four genes, we identified a number of gene variants, some of which specifically present in CF patients, suggesting a possible role of SLC26A genes in the pathogenesis of CF.
Acknowledgements: Grants from the "Fondazione per la ricerca sulla Fibrosi Cistica (Verona)" is gratefully acknowledged.

P21 INVESTIGATION OF CFTR ESONIC REARRANGEMENTS IN INFERTILE COUPLES

S. De Toffol, B. Malvestiti, F. Dulcetti, A. Ruggeri, A. Maggioni, M. Di Lernia, F. Maggi, G. Simoni, F.R. Grati. *Research and Development, Cytogenetics and Molecular Biology, TOMA Advanced Biomedical Assays S.p.A., Busto Arsizio, Varese, Italy*

Cystic Fibrosis (CF) is the most common monogenic autosomal recessive disease in Caucasians. In Italy, couples approaching assisted reproduction techniques (ART) are usually subjected to the first level CFTR mutation screening due to the high frequency of CFTR carriers in general population (1/27, 3.7%); in addition, men with congenital bilateral absence of vas deferens (CBAVD) and those with alterations in spermatogenesis present CFTR mutations (70–88% and 7%, respectively). Large CFTR gene rearrangements account for 1.5% of frequency in CF patients with classical manifestations and 1% in CBAVD men.
The aim of this work was to understand if CFTR esonic rearrangements could be a further possible cause of infertility.
Twenty-seven WT partners, belonging to discrepant couples after CFTR mutation screening and scanning, were investigated for the presence of esonic rearrangements by using QMPSF and MLPA analyses. In this preliminary study no large esonic rearrangements were found. Our future perspectives are to add the esonic rearrangements analysis to the first level mutation screening in order to investigate a larger cohort of patients.

P22 EMERGING PATHOGENS IN CYSTIC FIBROSIS: TEN YEARS FOLLOW UP

L. Spicuzza, C. Sciuto, G. Di Dio, L. Bivona, G. Cassisi, M. La Rosa. *Broncopneumologia Pediatrica, Dipartimento di Pediatria, Università di Catania, Catania, Italy*

Cystic fibrosis (CF) is characterized by specific age-related pathogens. Among emerging pathogens in recent years a higher prevalence has been found for *Burkholderia cepacia, Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans.*
Aim of our retrospective study was to establish the prevalence of CF commonly associated and emerging pathogens in a cohort of patients followed in our Cystic Fibrosis Unit for ten years. We studied a total of 109 patients (51 males, 42% children, 58% adults) followed in our Unit from January 1996 to December 2006. For each patient clinical records were examined, with microbiological findings and respiratory functional data. The highest prevalence of *Ps.aeruginosa*, was observed in 2004 (58%), followed by a clear decrease in the following years with the lowest rate recorded in 2006 (40.3%). The prevalence of *St.aureus* showed a highly irregular trend. The lowest prevalence was observed in 2004 (24%), with an increase recorded in the following years, with a maximum value recorded in 2006 (63%). We observed a clear increase in the prevalence of *B. cepacia* in the biennium 1996–1998 (13%) with a successive reduction to 5% in 2006. First isolations of *A.Xylosoxidans* were observed in 2002–2004 with a maximal prevalence of 3% and a reduction in the following years. None of the patients had a positive sputum for *S. maltophilia* until 2002. The prevalence of this pathogen progressively increased to 8% in 2004. In our population *B.Cepacia* has been associated to more severe clinical conditions characterised by a more rapid decline in FEV1 (mean FEV1 = 45.7%) and a rapid progression to respiratory failure (occurring in 25% of the patients with *B.Cepacia*). *S.Maltophilia* exhibited a mild effect on respiratory function (mean FEV1= 67%) and respiratory failure was present in 16.7% of the patients.
Generally, emerging pathogens have been observed only in the adult population.

P23 MOLECULAR EPIDEMIOLOGY OF *STENOTROPHOMONAS MALTOPHILIA* (SM) IN THE CYSTIC FIBROSIS (CF) CENTRE OF GENOVA

M. Mentasti, P. Morelli, G. Melioli, G. Manno. *General Laboratory of Analysis, "Giannina Gaslini" Institute, Genova, Italy*

Background: SM (previously known as *Pseudomonas maltophilia* and then as *Xanthomonas maltophilia*) is a non-fermentative, oxidase negative, Gram negative bacillus, intrinsically resistant to carbapenems, virtually resistant to all beta-lactams and often multiresistant. It has emerged as an important pathogen, particularly in immunocompromised patients and it is also frequently isolated from airway samples of CF patients; anyway its pathogenic role still remains not clearly understood.
Aim: The purpose of this study is to investigate if a patient to patient transmission and/or an environmental acquisition has occurred among CF patients in follow up at the CF centre of "Giannina Gaslini" Institute, Genova.
Materials and Methods: Since June 2004 a large collection of SM non-repetitive strains has been set up at the General Laboratory of Analysis, it comprises 91 strains isolated from 91/220 (41.4%) CF patients chronically/intermittently/sporadically colonized, 39 strains from non-CF patients and 25 strains from environmental sources (both hospital and community environment) for a total of 115 strains. 22/91 strains isolated from CF patients have been preliminarily analyzed. Genetic fingerprinting was performed by BOX-PCR according the protocol published by Rademaker J.L. *et al* in 1998 using BOX-A1R primer. SM ATCC 13637 was used as quality control strain. Cluster analysis was performed by Gel Compare II using Pearson's coefficient.
Results: BOX PCR analysis generated a good resolution of molecular profiles, allowing to perform an efficient analysis by Gel Compare II. Among the 22 CF analyzed strains a total of 12 different genotypes were found: 6 (50%) were not shared among patients while the other 6 (50%) were shared by 3 groups of 2 patients, 1 group of 3 patients and 1 group of 4 patients.
Conclusions: BOX PCR is a suitable method to determine the genetic relatedness of SM from CF patients. Although these are preliminary results, the data show clearly that SM seems to easily circulate among patients, as one half of the analysed strains are shared. Moreover, for a better comprehension, besides analysing the remaining CF strains it will be fundamental to analyze the non-CF and the environmental strains in order to establish if highly transmissible strains are circulating inside the hospital and/or an environmental acquisition has occurred.

P24 EMERGENCE OF COLISTIN-RESISTANT PSEUDOMONAS AERUGINOSA FROM ITALIAN CYSTIC FIBROSIS PATIENTS

G. Manno[1], P. Morelli[1], M. Mentasti[1], R. Casciaro[2], G.M. Rossolini[3], N. Cirilli[4], R. Gagliaridini[4], A. D'Aprile[5], F. Gioffrè[6], D. Scuteri[6], G. Melioli[1]. [1]*Microbiology Lab.,* [2]*Cystic Fibrosis Centre – Gaslini Genova,* [3]*University of Siena,* [4]*Cystic Fibrosis Centre Ancona,* [5]*Microbiology Lab. Cerignola,* [6]*Microbiology Lab. Soverato, Italy*

Background: Colistin (CL) has emerged as a relevant therapeutic option for the treatment of *P. aeruginosa* (PA) pulmonary infection in CF. The development of resistance to this agent under selective pressure is not unexpected, even if it may occur at lower extent compared with other antipseudomonal agents.
Objective: Monitoring the susceptibility to CL of a large collection of PA recovered from Italian CF pts.
Methods: During 2006, 295 PA strains (151 non-mucoid and 113 mucoid and 31 SCV) from 178 pts from 4 Italian CF Centres (Genova, Ancona, Cerignola and Soverato), were tested by CLSI disk diffusion method in Mueller–Hinton (MH) agar (BD) for 12 antipseudomonal drugs and CL using a 10 mcg disk. (BD) with PA 27853 ATCC as QC.
Isolates showing reduced susceptibility or resistance to CL disk were re-tested by Etest method in MH agar, after at least 10 repeated sub-culture in blood agar. The genetic relatedness of all isolates collection was previously determined by BOX-PCR.
Results: Four PA isolates (1 mucoid, 1 non-mucoid and 2 SCV) from 4 patients (3 from Genova and 1 from Ancona) showed resistance to CL by disk diffusion and confirmed by Etest. The resistance level was high, with the Minimum Inhibitory Concentration (MIC) ranging from 12 to 32 mcg/ml. The 2 SCV strains were multi-drug-resistant, while the mucoid isolate was susceptible to the other antipseudomonal drugs tested. The CL-resistant PA isolates showed different genetic profiles by BOX-PCR. 3/4 patients chronically colonized by CL-resistant PA had several previous antibiotic treatments with aerosolised CL.